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Effects of Two Physical Training Paradigms on Biological and Cognitive Characteristics of Airmen: Lessons Learned

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14. ABSTRACT In order to determine the effect of different physical training paradigms on Airman biological and cognitive profiles over time, a complex exercise-based pilot experimental program was conducted over a 12-week period. During that time, Active Duty participants engaged in a 9-week physical training program, tests of physical endurance and strength, body composition analyses, biomarker collections, and cognitive tests. In addition to briefly discussing results, this paper provides a current review of exercise-induced brain derived neurotrophic factor (BDNF) literature and discusses the lessons learned for conducting future experiments of this nature. It focuses on how to refine experimental designs of future exercise-based AF research efforts, addressing issues associated with Active Duty Airmen subjects such as attrition and controls.

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1.0 SUMMARY

In order to determine the effect of different physical training paradigms on Airman biological and cognitive profiles over time, a complex exercise-based pilot experimental program was conducted over a 12-week period. During that time, Active Duty participants engaged in a 9-week physical training program, tests of physical endurance and strength, body composition analyses, biomarker collections, and cognitive tests. In addition to briefly discussing results, this paper provides a brief current review of exercise-induced brain derived neurotrophic factor (BDNF) literature and discusses the lessons learned for conducting future experiments of this nature. It focuses on how to refine experimental designs of future exercise-based AF research efforts, addressing issues associated with Active Duty Airmen subjects such as attrition and controls.

2.0 INTRODUCTION

It is well-known that physical fitness is a key attribute to our military, however due to the complex environments that Airmen work in, it is imperative to optimize cognitive readiness, as well as the physical fitness of Airmen to ensured mission success.

Recent research has investigated the changes in circulating Brain-Derived Neurotrophic Factor (BDNF) levels associated with physical activity, which may positively affect cognitive processes through improved lobe and synaptic connectivity (Voss et al., 2013). Numerous results in the literature have supported the potential connection between BDNF levels and improved cognitive capacity. For example, Gliogoroska and Manchevska (2012) reviewed physiological mechanisms that influence cognition, including pathways that are directly influenced by BDNF. Also, Mooren and Volker's (2005) results indicated that 'BDNF infusions enhance learning, while a BDNF deficiency disrupts learning'. Circulating levels of BDNF have also been associated with hippocampal function and volume. Erickson et al., (2010) discovered a relationship between plasma BDNF levels, hippocampal volume, and cognitive performance which indicated that lower BDNF levels were associated with smaller hippocampal volume, and decreased memory. Since the completion of this research effort, Skriver et al., (2014) demonstrated a strong correlation between plasma BDNF levels and improved retention of motor skills following a single bout of aerobic exercise.

Given these results it was clear that understanding the link between BDNF and cognitive performance could potentially inform the refinement of Airmen training paradigms to optimize both physical fitness and cognitive readiness. To properly assess the Air Force relevance of BDNF results to improving training, a new laboratory capability within RHCP was needed, coupled with an initial operational pilot test of that capability.

To accomplish this, a new human performance laboratory capability was stood up, and BDNF sample collection and analysis procedures were developed. Then an initial pilot research study was to determine the influence of two different physical training paradigms on circulating BDNF concentrations and if the training paradigms influenced cognitive performance.

As a test paradigm a 'Cross-Fit-type' training program was selected, based on recommendations of retired Air Force Special Forces Operators, and contrasted against the traditional Air Force fitness training (AFFT, detailed in AFI 39-2905). It was hypothesized that BDNF levels would acutely increase following the VO2 max test, and chronically increase as the participants engaged in the 9-week training paradigm. Although it was expected that both training paradigms would increase scores on cognitive performance tasks, it was hypothesized that 'CrossFit-type' training would be more effective at producing chronically elevated BDNF levels and fitness scores than the traditional AF fitness training and that the improvements in cognitive performance would be correlated with changes in BDNF levels.

Specifically, the objectives of the pilot research were stand up and test a new laboratory capability to determine: 1) if BDNF levels were increased acutely after maximal exercise; 2) if baseline BDNF levels increased and were maintained over time as subjects participated in a prolonged exercise program; and 3) if changes in BDNF correlated with improved scores on the cognitive tasks.

Training for improved cognitive performance would have important potential Air Force applications. Therefore, given the previous research results relating exercise-induced BDNF levels with improved cognition, it is important to test the relationship in Air Force relevant settings.

3.0 METHODS, ASSUMPTIONS, and PROCEDURES

3.1 Participants

Eighteen Active Duty male subjects, ages ranging from 18-40, participated in this pilot research effort. Subjects were divided into two groups: Nine in 'CrossFit - type' training and nine subjects matched for fitness, age, height, and weight in the traditional Air Force fitness training group. Certified CrossFit trainers implemented the CrossFit-Type exercises. Of note, each group ultimately contained 6 subjects instead of nine original subjects. This was due to attrition or low attendance throughout the study. Data from subjects who missed more than 30% of the 36 workout sessions were removed from analysis.

3.2 Procedures & Metrics

Subjects underwent baseline physiological, BDNF profiling, and cognitive assessments followed by 9 weeks of physical training, periodic cognitive assessments, and periodic blood draws for circulating BDNF. Post-test assessments similar to baseline were performed at the conclusion of the training paradigms (See Fig.1 for Experimental Timeline).

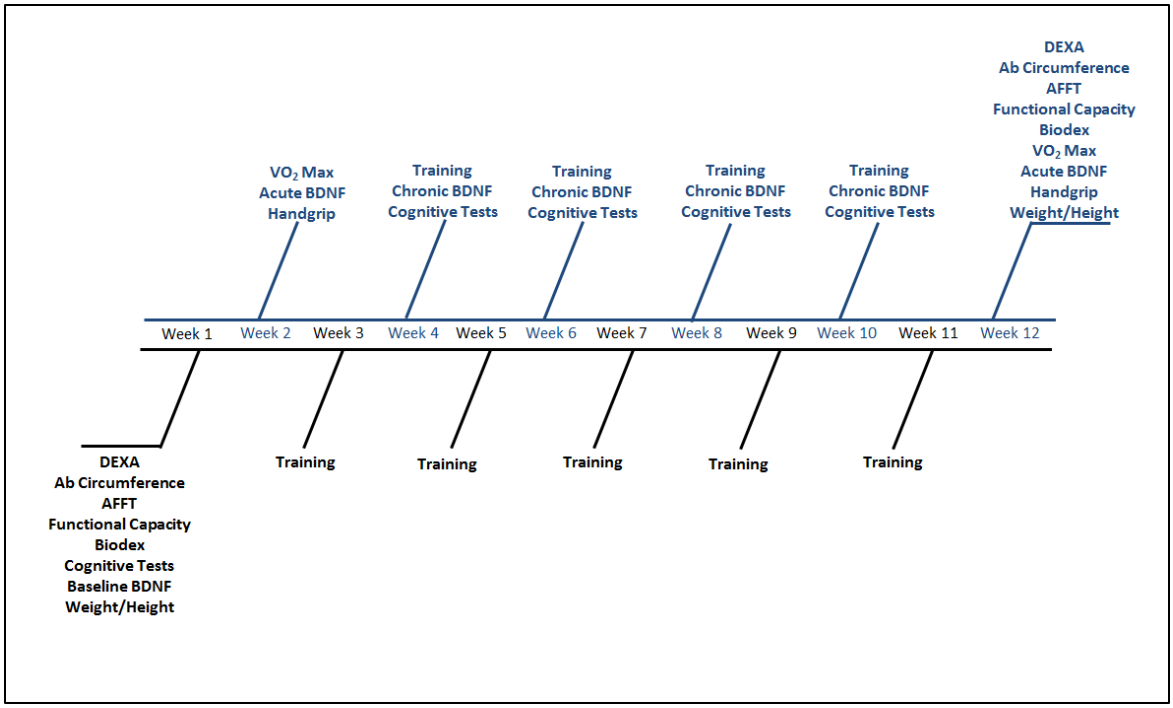

Figure 1. Experimental Timeline

3.2.1 Physiological Metrics

During weeks 1, 2 and 12 of this effort, physiological metrics were collected on each participant. The following assessments were performed.

3.2.1.1 Dual-energy x-ray absorptiometry (DEXA)

To measure body composition, percent fat and lean mass, a GE DEXA machine (Prodigy Lunar DXA, Model 8743, GE Healthcare Encore) was utilized. Subjects were scanned with a minimal dose of x-ray to obtain body composition data.

3.2.1.2 Air Force Fitness Test

AFI 39-2905 was followed to measure fitness levels among participants. Tasks completed to mimic this test included a timed 1.5 mile run, abdominal circumference measurements, and 1-minute timed push-ups and sit ups. The abdominal circumference measurement was performed by utilizing a Gulick anthropometric measuring tape and including a constant tension system that prevents overtightening. The tape was placed lightly around the bare skin of the subject's abdomen. The tape contacted the skin without indenting the skin or compressing fatty tissue. Measurements were taken on a horizontal plane just above the uppermost border of the iliac crests.

3.2.1.3 Functional Capacity (FC)

To represent operationally-relevant tasks which require strength and power, the functional capacity test defined by Williams and Rayson (2006), Williams, Williams, and Evans (2007), and Williams and Wilkinson (2007) was used. This

task included subjects repetitively lifting and carrying a 22-kg sandbag 10 meters and placing it on a platform (based on the height of a truck (1.45 m) to be loaded) then returning it to the start position. Subjects had 10 minutes to complete the task. The number of full repetitions completed in that 10 minutes was recorded.

3.2.1.4 Isokinetic BioDex

An isokinetic BioDex dynamometer was used to measure thigh muscular strength (ST), endurance (E), and muscular power output (PO). The evaluation of knee extension and flexion occurred because knee extension and flexion represents the strength of the quadriceps and hamstrings musculature (Adams, 1991 & 1998). This assessment represented the ST, E, and PO of four quadriceps muscles to include, rectus femoris, vastus medialis, vastus lateralis, and vastus intermedius, and hamstring muscles to include, biceps femoris, semitendinosus, and semi membranosus. Maximal effort was encouraged. Tests were conducted at 60, 180, and 300 degrees per second with a 3 minute rest between tests. Three trials were completed at each frequency. Highest torque generated for each group of 3 trials was recorded.

3.2.1.5 Handgrip

To measure handgrip strength, a handgrip dynamometer was utilized. Measurements from this device were used to represent upper body strength. Two to three trials were completed alternately with each hand with instructions to squeeze the device maximally and quickly. The best (maximal) score was recorded.

3.2.1.6 VO2 Max

Each subject's maximum oxygen uptake volume (VO2) was determined on a Woodway Desmo treadmill using a modified Bruce exercise protocol. In order to evaluate expired breathing gases, subjects breathed into a fitted mask with a one-way respiratory breathing valve connected to a metabolic cart. A metabolic cart, using open circuit spirometry, was used to measure maximal aerobic fitness level (Adams, 1991 & 1998). Subjects performed the protocol while respiratory quotient (RQ), heart rate, and perceived exertion was monitored until volitional fatigue.

3.2.1.7 Weight/Height

For weight, a calibrated electronic scale was utilized. Subjects stood barefoot on the scale and wore similar clothing for the pre/post measurements. Participants were measured for height using a stadiometer.

3.2.2 Cognitive Metrics

During weeks 1, 4, 6, 8, and 10 computer-based cognitive tests were administered to determine cognitive function pre and post training session. The following tests were administered to evaluate attention, short term memory and vigilance.

3.2.2.1 Dichotic Listening Test

The goal of this test was to evaluate attention and required the participant to attend to auditory information presented in one ear (the “attending” ear) while at the same time, distracting information being delivered to the "non-attending" ear. The subject responds accordingly and answers are collected and stored.

3.2.2.2 Continuous Memory: N-Back test

This task indexes the operator’s ability to encode and store information in working, or “short-term” memory. It requires serial encoding and recall under a changing memory state.

3.2.2.3 Visual Vigilance

Participants were required to monitor a particular object over a 12-minute task and stay engaged during mundane tasks. General principles developed by Temple et al., (2000) were followed during this experiment.

3.2.3 Biomarker Metrics

3.2.3.1 Acute BDNF

Baseline samples were obtained as subjects entered the program before any exercise. A second set of samples were collected during weeks 2 and 12. BDNF levels were measured in serum collected via venipuncture immediately following the completion of the VO2 maximum test. These levels were considered ‘acute’ measures as they were assessed immediately following an intense physical exertion. Subjects were escorted to the medical monitor room where blood samples were collected within 5 minutes of completion of the VO2 max test. For each sample, three mL of blood was collected into a serum separator tube (SST) and allowed to clot for 30 min at room temperature. Tubes were centrifuged 15 min at 1000 x g. Serum was removed, separated into 200µL aliquots, and stored at -80 degrees C.

3.2.3.2 Chronic BDNF

During weeks 1, 4, 6, 8, 10, BDNF levels were measured in serum collected via venipuncture before training. These levels were consider ‘chronic’ measures as they were collected during a baseline state. Samples were collected on Thursday mornings prior to the initiation of the work-out for that day. These samples were intended to provide a “snapshot” view of BDNF levels in response to training accomplished in that period. Thursdays were selected because the training paradigms occurred on Monday, Tuesday, Thursday and Friday each week. Therefore to obtain a true chronic value after a day of rest, Thursdays were selected. For each sample, three ml of blood was collected into a serum separator tube (SST) and allowed to clot for 30 min at room temperature. Tubes were centrifuged 15 min at 1000 x g. Serum was removed, separated into 200µL aliquots, and stored at -80 degrees C.

3.2.3.3 Acute & Chronic BDNF Analyses

All samples were thawed on wet ice to room temperature prior to start of assay. Enzyme-Linked Immunosorbent Assay (ELISA) techniques were used to determine levels of total circulating serum BDNF (DBD00; R&D Systems, Minneapolis, MN). Except for diluting serum samples 1:30, the manufacturer's protocol was followed and a standard curve (0-2000pg/mL) fitting a 4-parametric function was used to calculate BDNF concentration.

4.0 RESULTS

4.1 Pre to Post t-tests by Training Group

Two-tailed t-tests were completed to uncover pre to post testing changes within subjects and between groups for all measures. There were three main categories of dependent measures analyzed: Traditional physiological measures, BDNF levels, and cognitive performance measures.

4.1.1 Traditional Physiological Measures

The effect of the two training programs on tradition physiological measures of body dimensions, strength, and cardiovascular fitness are presented in Table 1. Physiological measures that significantly changed in both exercise groups were percent body fat, percent lean mass, FC reps, and VO2 Max. Body weight, abdominal circumference, and left handgrip max were significantly different in the 'CrossFit - type' group but not the AFFT group. The AFFT 1.5 mile Run time was significantly different from pre and post in the traditional training group and not the 'CrossFit - type'. Improvements in these physiological parameters were expected due to the fact that both training regimes were designed to explicitly improve physical fitness.

Table 1. Two-tailed t-tests for significance of mean change from pre to post for each training group's traditional physiological measures

	Traditional (n = 6)					'CrossFit - type' (n = 6)				
	Mean ± SEM			t-test	% Pre	Mean ± SEM			t-test	% Pre
Variable	Pre	Post	Pre to Post	p	to Post	Pre	Post	Pre to Post	p	to Post
Body Weight (lbs)	193.2 ± 15.1	192.4 ± 14.0	-0.8 ± 1.4	0.5864	-0.4	197.1 ± 11.8	189.9 ± 10.8	-7.3 ± 1.8	0.0099	-3.7
Dexa Body Fat (%)	25.0 ± 2.9	23.2 ± 2.8	-1.8 ± 0.7	0.0432		25.0 ± 3.1	22.1 ± 3.5	-2.9 ± 0.6	0.0060	
Dexa Lean Mass (%)	74.8 ± 2.9	76.8 ± 2.9	2.0 ± 0.6	0.0180		75.0 ± 3.0	77.8 ± 3.6	2.8 ± 0.7	0.0075	
FC Number of Reps	29.2 ± 2.0	37.0 ± 2.2	7.8 ± 0.8	0.0002	26.9	30.0 ± 2.4	36.0 ± 2.3	6.0 ± 1.2	0.0035	20.0
VO2 Max VO2 (mL/kg/min)	44.9 ± 2.1	49.1 ± 1.9	4.2 ± 1.0	0.0075	9.2	46.5 ± 2.5	50.7 ± 3.0	4.2 ± 1.1	0.0123	9.0
VO2 Max Heart Rate (bpm)	183.3 ± 4.6	177.5 ± 1.8	-5.8 ± 3.2	0.1297	-3.2	193.3 ± 2.1	191.5 ± 5.2	-1.8 ± 5.9	0.7697	-0.9
VO2 Max RER	1.12 ± 0.03	1.12 ± 0.03	0.00 ± 0.04	0.9123	0.45	1.14 ± 0.03	1.13 ± 0.03	-0.01 ± 0.05	0.7770	-1.31
Ab Circumference (in)	36.7 ± 1.7	35.5 ± 1.4	-1.2 ± 0.6	0.0923	-3.2	36.3 ± 1.1	34.5 ± 1.2	-1.9 ± 0.4	0.0084	-5.1
AFFT Run (min)	12.4 ± 0.6	11.4 ± 0.3	-1.0 ± 0.3	0.0181	-8.0	11.8 ± 0.7	11.0 ± 0.5	-0.8 ± 0.3	0.0572	-7.1
AFFT Number of Pushups	47.2 ± 3.3	48.7 ± 3.1	1.5 ± 1.1	0.2480	3.2	45.8 ± 4.9	45.4 ± 4.1	-0.4 ± 2.5	0.8807	-0.9
AFFT Number of Sit-ups	51.5 ± 4.3	52.5 ± 2.1	1.0 ± 3.2	0.7683	1.9	51.8 ± 3.4	54.8 ± 3.8	3.0 ± 1.4	0.0951	5.8
Handgrip Max Right (kg)	54.0 ± 4.4	61.3 ± 4.9	7.3 ± 3.1	0.0666	13.6	53.0 ± 4.1	57.2 ± 1.9	4.2 ± 3.0	0.2284	7.9
Handgrip Max Left (kg)	53.0 ± 3.7	58.5 ± 4.9	5.5 ± 2.5	0.0814	10.4	48.0 ± 2.2	55.6 ± 1.7	7.6 ± 1.9	0.0165	15.8
Peak TQ/BW Right (%)	78.9 ± 7.5	84.2 ± 4.8	5.3 ± 5.6	0.3913		90.1 ± 7.3	95.0 ± 5.2	4.9 ± 4.2	0.2961	
Peak TQ/BW Left (%)	79.4 ± 6.2	85.4 ± 6.7	6.0 ± 3.8	0.1762		84.1 ± 7.4	84.7 ± 5.4	0.6 ± 6.1	0.9238	

4.1.2 BDNF Levels

The effect of the two training programs on BDNF levels are presented in Table 2. None of the BDNF t-tests reached significance in either the Traditional or the Crossfit groups.

Table 2. Two-tailed t-tests for significance of mean change from pre to post for each training group's BDNF Levels

	Traditional (n = 6)					'CrossFit - type' (n = 6)				
	Mean ± SEM			t-test	% Pre	Mean ± SEM			t-test	% Pre
Variable	Pre	Post	Pre to Post	p	to Post	Pre	Post	Pre to Post	p	to Post
BDNF (pg/mL x1000) pre exercise	15.6 ± 1.2	16.4 ± 1.7	0.8 ± 1.5	0.6180	5.2	17.2 ± 1.7	16.6 ± 1.4	-0.6 ± 1.5	0.7200	-3.5
BDNF (pg/mL x1000) post exercise	22.3 ± 1.1	20.2 ± 1.7	-2.1 ± 1.7	0.2719	-9.3	26.5 ± 2.2	23.2 ± 1.9	-3.3 ± 1.5	0.0848	-12.4

4.1.3 Cognitive Tests

The effect of the two training programs on cognitive test scores are presented in Table 3. Subjects often achieved maximum achievable score on the cognitive tests. None of the cognitive performance t-tests reached significance in either the Traditional or the Crossfit groups.

Table 3. Two-tailed t-tests for significance of mean change from pre to post for each training group's performance on the cognitive tests. (CM: Continuous Memory, VV: Visual Vigilance, DL: Dichotic Listening)

	Traditional (n = 6)					'CrossFit - type' (n = 6)				
	Mean ± SEM			t-test		Mean ± SEM			t-test	
Variable	Pre	Post	Pre to Post	p		Pre	Post	Pre to Post	p	
CM (% correct)	86.5 ± 7.6	90.5 ± 7.9	4.0 ± 2.7	0.2075		90.2 ± 3.3	95.8 ± 1.3	5.6 ± 3.2	0.1451	
VV (% correct)	98.4 ± 0.7	97.4 ± 1.3	-1.0 ± 1.5	0.5290		96.4 ± 1.5	99.0 ± 0.7	2.6 ± 1.9	0.2245	
DL (% correct)	95.8 ± 2.0	97.2 ± 1.4	1.4 ± 2.8	0.6351		87.5 ± 0.6	91.7 ± 2.7	4.2 ± 2.8	0.1908	

4.2 Pre to Post t-tests across Training Groups

To increase statistical power, the data from the training groups were combined to determine if any changes in BDNF levels and physiological parameters from pre to post testing were detectable (Table 4).

'BDNF Pre exercise' indicates samples collected during week 1 (baseline), 4, 6, 8, and 10, while 'BDNF post exercise' were samples collected in weeks 2 and 12 (post VO2 max). Physiological markers were compared from week 1 or 2 (pre) and week 12 (post). The only measures that changed significantly in this pooled sample were bodyweight, and handgrip strength for both hands.

Analyses of variance were performed using weeks 1 & 2 in one analysis and weeks 10 & 12 in a separate analysis to compare pre to post VO2 max. The dependent variable was BDNF with between factor group and within factor week. Cohen's *d* is given as an effect size (value of 0.8 or larger implies a possible large effect). There was a significant change (+8.5) from week 1 to week 2 [$F(1, 10) = 19.83$, $p = 0.0012$] and a significant change (+5.2) from week 10 to week 12

[F (1, 10) = 17.99, p = 0.0022]. There was not a group*week interaction for either analysis (p > 0.3748).

Table 4. Two-tailed two-sample t-tests for mean change from pre-to post

Variable	Change from Pre to Post Mean ± SEM Traditional	Change from Pre to Post Mean ± SEM Crossfit	Mean Diff	Two-Tailed Two-Sample t-test DF	t	p	Cohen's d	ANOCOV LSMean Trad	Cross	p
BDNF (pg/mL x1000) pre exercise	0.8 ± 1.5	-0.6 ± 1.5	1.4	9	-0.64	0.5368	0.43	0.5	-0.3	0.7632
BDNF (pg/mL x1000) post exercise	-2.1 ± 1.7	-3.3 ± 1.5	1.2	10	-0.52	0.6111	0.33	-2.8	-2.6	0.9551
CM (% correct)	4.0 ± 2.7	5.6 ± 3.2	-1.6	10	0.38	0.7153	0.24	5.9	3.7	0.6074
VV (% correct)	-1.0 ± 1.5	2.6 ± 1.9	-3.6	10	1.50	0.1646	0.95	-0.3	1.9	0.4292
DL (% correct)	1.4 ± 2.8	4.2 ± 2.8	-2.8	10	0.71	0.4924	0.45	3.1	2.5	0.8815
Body Weight (lbs)	-0.8 ± 1.4	-7.3 ± 1.8	6.5	10	-2.83	0.0180	1.79	-1.0	-7.1	0.0494
Dexa Body Fat (%)	-1.8 ± 0.7	-2.9 ± 0.6	1.1	10	-1.19	0.2607	0.75	-1.6	-3.1	0.1705
Dexa Lean Mass (%)	2.0 ± 0.6	2.8 ± 0.7	-0.8	10	0.96	0.3620	0.60	1.8	3.0	0.2752
FC Number of Reps	7.8 ± 0.8	6.0 ± 1.2	1.8	10	-1.31	0.2179	0.83	7.4	6.4	0.5119
VO2 Max VO2 (mL/kg/min)	4.2 ± 1.0	4.2 ± 1.1	0.0	10	0.01	0.9911	0.01	3.3	5.0	0.1949
VO2 Max Heart Rate (bpm)	-5.8 ± 3.2	-1.8 ± 5.9	-4.0	10	0.59	0.5664	0.37	-5.4	-2.3	0.7013
VO2 Max RER	0.00 ± 0.04	-0.01 ± 0.05	0.02	10	-0.30	0.7688	0.19	0.00	0.02	0.5488
Ab Circumference (in)	-1.2 ± 0.6	-1.9 ± 0.4	0.7	10	-0.97	0.3558	0.61	-1.1	-1.9	0.3687
AFFT Run (min)	-1.0 ± 0.3	-0.8 ± 0.3	-0.1	9	0.35	0.7370	0.23	-0.7	-1.2	0.2354
AFFT Number of Pushups	1.5 ± 1.1	-0.4 ± 2.5	1.9	9	-0.73	0.4819	0.49	1.7	-0.7	0.4981
AFFT Number of Situps	1.0 ± 3.2	3.0 ± 1.4	-2.0	9	0.53	0.6082	0.36	-1.1	5.5	0.1440
Handgrip Max Right (kg)	7.3 ± 3.1	4.2 ± 3.0	3.1	9	-0.72	0.4922	0.48	10.4	0.5	0.0380
Handgrip Max Left (kg)	5.5 ± 2.5	7.6 ± 1.9	-2.1	9	0.64	0.5384	0.43	8.5	4.1	0.0262
Peak TQ/BW Right (%)	5.3 ± 5.6	4.9 ± 4.2	0.4	10	-0.05	0.9592	0.03	6.4	3.7	0.7474
Peak TQ/BW Left (%)	6.0 ± 3.8	0.6 ± 6.1	5.4	10	-0.75	0.4699	0.47	7.4	-0.7	0.3556

4.3 Analysis of BDNF throughout training

Blood draws were taken prior to workout session to assess 'chronic' changes in BDNF. Serum BDNF levels in weeks 2, 4, 6, 8, and 10 were compared to week 1 (Table 5). Week 2 indicated an increase from the previous week, however no significant changes were observed in the subsequent weeks.

Table 5. Analyses of BDNF variance across study. Significance was based off p-value<0.05 and highlighted in gray

Week	Group	BDNF Change from Week1 Mean	SEM	Two-Tailed Paired t-test n	t	p	n Non-Para	Wilcoxon Signed Rank p	Fisher's Sign test B	p
2	CrossFit	10.25	3.57	6	2.87	0.0348	6	0.0313	6	0.0313
	Traditional	6.71	1.33	6	5.03	0.0040	6	0.0313	6	0.0313
4	CrossFit	3.45	2.54	6	1.36	0.2326	6	0.3125	4	0.6875
	Traditional	-0.71	1.57	6	-0.45	0.6728	6	0.5625	4	0.6875
6	CrossFit	2.27	2.24	6	1.01	0.3569	6	0.6875	3	1.0000
	Traditional	0.61	1.18	6	0.51	0.6304	6	1.0000	3	1.0000
8	CrossFit	0.12	1.46	5	0.08	0.9372	5	1.0000	3	1.0000
	Traditional	1.58	1.9	6	0.83	0.4445	6	0.6875	3	1.0000
10	CrossFit	-0.59	1.54	5	-0.38	0.7200	5	0.6250	3	1.0000
	Traditional	0.8	1.51	6	0.53	0.6180	6	0.8438	3	1.0000
12	CrossFit	6.97	2.75	6	2.53	0.0523	6	0.0313	6	0.0313
	Traditional	4.63	2.36	6	1.96	0.1068	6	0.0938	5	0.2188

5.0 DISCUSSION

Although this was a pilot project, many of the goals of the study were achieved. This pilot study was a low-cost way to build and validate the BDNF analysis process, and techniques were developed to detect changing levels of BDNF after exercise. This study laid the ground work for further work in the area of physical training and cognitive performance.

Results comparing BDNF levels at time points before and after V02 max support literature that found BDNF increases following acute exercise (Skriver et al., 2014). The results did not, however, produce the anticipated change in body weight due to following a regimented workout routine (Levinger et al., 2008). The results also did not support the hypothesis that 'CrossFit - type' fitness paradigms are more effective than traditional routines at improving physical, cognitive or BDNF metrics. Additionally, BDNF levels were not elevated chronically due to exercise. No association between BDNF and cognitive performance was demonstrated in this project.

However, the limited results of this research were potentially due to experimental design and procedural limitations in this pilot study, and evaluation of this initial pilot study can lay the ground work for further work in the area of physical training and cognitive performance. Numerous 'Lessons Learned' resulted from this work.

5.1 Lessons Learned: Subject Participation

5.1.1 Participant Data Inclusion Criteria

This study had the challenge of running Active Duty participants who often have scheduling conflicts such as temporary duty assignments (TDYs) and even permanent change in station (PCS) moves causing them to miss scheduled training sessions or even leave the study completely. In order to reduce variance among subjects, a workout attendance record of 90% would have been ideal, however, if the exclusion criteria was set at 90% attendance, only 2-3 participants would have been left in each group resulting in the inability to conduct proper statistical analysis. Therefore, data from subjects who completed 70% or more of the training, a number that might be expected in realistic real-world exercising, were included in the analysis, resulting in 6 subjects per group which significantly affected the statistical analysis. For subsequent studies of a similar population and duration, the subject recruitment goal should be at least 50% higher than required for statistical analysis to properly account for attrition.

5.1.2 Participant Incentive

Subject participation was one of the major constraints of the study. To overcome the lack of motivation for completing a 12-week research effort, one way forward is to incentivize the subjects. This could be in the form of coins, fitness gear, improved PT scores, day-passes, or other forms of reimbursement approved for military participants.

5.1.3 Participant Inclusion Criteria

The inclusion criteria for this initial study were male Active Duty participants who were: not on medical profile, did not pose a risk for cardiovascular disease (must not be

prescribed blood pressure medications or herbal dietary supplements that could affect heart rate response), were free of musculoskeletal injuries, had supervisor approval and were available to participate for 12 consecutive weeks. This study did not take into account participants suffering from depression/anxiety disorders or those taking medication for treatment. This poses a problem in data analysis as there are known correlations between low BDNF levels in people diagnosed with major depression disorder (MDD) (Brunoni, Lopes & Fregni, 2008). Research has shown that pharmaceutical treatments for depression, for example, serotonin reuptake inhibitors (SSRI's), elevate BDNF levels (Schimizu et al., 2003 and Brunoni et al., 2008) to compensate for that loss; which could affect the results of the research without the investigators being aware. It is to be noted that all Active Duty members participating in this study were most likely not on medical profile, therefore not suffering from major depression disorder; although it is a factor to consider when conducting studies examining BDNF. It is recommended in follow-on efforts that questions regarding mental health status and medication history be included in the inclusion criteria to avoid participation from individuals who may have compromised BDNF levels.

5.2 Lessons Learned: Controls

This study was comprised of two experimental groups: the 'traditional' Air Force training regimen and 'non-traditional' 'CrossFit - type' training regimen. Neither regimen produced a significant effect in chronic BDNF over the course of the study, although as previously noted the results were limited given the subject attrition. The literature suggests a need for sedentary controls when evaluating differences in exercise-induced BDNF levels, as quoted from Schmolesky, Web, and Hansen (2013), 'Having sedentary controls within the laboratory setting proved to be an important approach to this study, as there was an unexpected but consistent pre-post decrease in serum-BDNF (sBDNF) levels among controls on the order of 13%. Thus, subjects in the exercise condition actually demonstrated an approximate 45% increase in sBDNF levels relative to sedentary controls'. While sedentary controls would have been the appropriate 'control group,' the subject population for this effort was Active Duty males, all of which had Physical Training (PT) requirements to meet on an annual basis. The rationale for conducting the pilot study without a true control group was because for this population, traditional PT is the baseline condition as it is a requirement. The only population within the Air Force that may have served in this fashion would be those on medical profile whom do not require PT, however, due to the varying reasons for being put on profile, BDNF levels may have been affected for that control group as well. Nonetheless, for future BDNF research efforts, a control group who is sedentary throughout the experiment would better elucidate chronic differences.

5.3 Lessons Learned: Training Intensity Effects on BDNF

Previous studies have reported that the intensity of workout affects the BDNF response following exercise. It has been demonstrated that high intensity workouts result in a significant increase in BDNF compared to baseline, whereas low intensity workouts do not (Schmidt-Kassow et al., 2012 Rojas Vega, Hollmann, Vera Wharmann, & Strüder, 2012; Ferris, Williams & Shien, 2006). This trend was not seen in resistance training, in which a single bout of high and low intensity training did not affect acute BDNF concentrations in either exercise group (Rojas Vega, Knicker, Hollmann, Bloch, & Strüder, 2010). The literature suggests that the type, duration, and intensity of exercise is important in whether a significant difference will be

exhibited in peripheral BDNF levels. Also, the literature reveals opposing evidence of what induces a significant rise in peripheral BDNF levels, therefore displaying the difficulty when designing a study involving circulating BDNF. Although the 'CrossFit - type' training was modeled after intensive training, levels of BDNF were not captured following that regiment, therefore these types of trends were not collected during this study. Future research efforts exploring chronic BDNF levels should therefore be assessed following high intensity workout regiments to identify trends.

5.4 Lessons Learned: Cognitive Test Battery

The cognitive tests that were chosen for the study consisted of Dichotic Listening Test, N-Back Test, and a Visual Vigilance task. These tests are often used to discern gross cognitive changes due to neurological deficits. The subject population were healthy active duty military members and performed at high levels across all tests. A ceiling effect was reached which therefore made it difficult to ascertain a difference from baseline and associations between performance and BDNF levels. The difficulties in seeing a difference in cognitive performance as indicated by the baseline results elucidates a need for more arduous tasks when the subject population is comprised of neurological healthy subjects. The difficulty levels of the batteries should have therefore been increased at the start of the study. In order to observe significant associations between BDNF and cognitive parameters, a plethora of cognitive tasks may be required to capture different types of cognition (learning, retention, vigilance, motor, attention, etc.). It is important to identify particularly demanding cognitive tasks in order to avoid a ceiling effect.

5.5 Lessons Learned: Blood Collection Timing and Methodology

5.5.1 Timing of Blood Collection

Due to the varying results of BDNF level elevations under the different parameters, this effort sought to determine if basal BDNF levels could be modified using various training paradigms. The blood collections were timed such that acute responses were captured within 5 minutes of VO2 Max completion. Previous studies have investigated peripheral BDNF duration mechanisms of action prior, during, and following a bout of aerobic exercise utilized an IV blood collection. Schmidt-Kassow et al., (2012) reported that BDNF concentration decreased after reaching a peak 15-20 min into high intensity exercise. The same study reported a return to baseline within approximately 10 min of recovery. Other studies have noted the narrow range of peripheral BDNF induction with cessation occurring from 5 min (Skriver et al., 2014) to 10 min (Schmidt-Kassow et al., 2012 and Rojas Vega, et al., 2012). The range of BDNF induction indicates the importance of the timing of blood draws in order to capture the after-effects of exercise.

'Chronic BDNF' blood draws were taken prior to workouts to track basal BDNF changes during weeks 1, 4, 6,8,10. The mechanism underlying basal and exercise induced BDNF release may involve different processes, as noted from Seifert et al., (2010). The literature shows the majority of chronic exercise studies do not observe significant changes in basal BDNF levels (Swift et al., 2012; Griffin et al., 2011; Goekint et al., 2010; Schiffer, Schulte, Hollman, Bloch & Struder, 2009; Levinger et al., 2008). The current study did not find significant effects in the chronic blood draws when compared to baseline at week 1. Since the majority of BDNF research suggests there may be a

change in BDNF induction (Griffin et al., 2011 and Ferris et al., 2006), not basal state, future investigations should utilize immediate post exercise collections. Seeing a steady increase in the induction of BDNF may be an indication of the ability of the body to produce BDNF more readily as individuals become more physically fit, which could lead to improved cognitive abilities. Our data did not reveal significant changes in basal BDNF concentrations, but that does not suggest a chronic induction of BDNF levels did not exist, or other benefits from the training regimens were not derived.

5.5.2 Site of Blood Collection

The blood collection site is an important factor to consider when determining appropriate BDNF levels. Researchers have measured BDNF levels when collecting blood from the jugular venous (Seifert et al., 2010). The same study found a difference between BDNF levels collected from jugular venous and brachial artery at rest and in response to exercise, indicating a difference in maintenance of BDNF at rest and during exercise. The reasoning to measure blood that is closer to the brain is the thought that peripheral venous blood may blunt the contribution of BDNF from the brain (Seifert et al., 2010 and Goekint et al., 2010). Other researchers have utilized the brachial vein to collect their blood samples (Rojas Vega et al., 2010), which is a little further up the arm than where blood is typically collected. The variance among the literature examining peripheral BDNF levels could be attributed to the location of collection site, which makes it harder to determine response of BDNF following exercise. Therefore, careful consideration should be made concerning blood collection location.

6.0 CONCLUSIONS

BDNF is an important biomarker of interest given its involvement in several physiological and cognitive systems. The study presented in this report was designed to examine the effects of physical training on serum BDNF levels and cognitive performance in active duty Airmen. The study successfully established a lab and data collection techniques for examining physical training effects on physiology and cognition. Further, the investigators searched for indications that prevailing levels of BDNF could build over time during an exercise program and potentially be utilized as an augmentation tool for skills training. This study also investigated whether differences in performance and biomarker parameters could be observed between traditional Air Force training and ‘Cross fit-type’ training. The results revealed limited significant findings due to the limitations of this pilot study. Acute serum BDNF response to exercise was observed following VO2 max. The findings of this study should be considered with the understanding of the limitations described. The data is therefore considered preliminary and support future physical training studies to better understand physical training effects on BDNF and ultimately cognitive performance. Taking into consideration the revisions recommended to the study design outlined in this report, combined with the recent evidence in the literature, future studies could help elucidate the associations between biomarkers and physical/cognitive performance in order to help maintain readiness in our Airmen.

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8.0 ACRONYMS LIST

AFFT	Air Force fitness training
BDNF	brain derived neurotrophic factor
CM	continuous memory
DEXA	dual-energy x-ray absorptiometry
DL	dichotic listening
E	endurance
ELISA	Enzyme-Linked Immunosorbent Assay
FC	functional capacity
MDD	major depression disorder
PCS	permanent change in station
PO	power output
RHCP	Applied Neuroscience Branch
RQ	respiratory quotient
sBDNF	serum brain derived neurotrophic factor
SST	serum separator tube
ST	strength
TDY	temporary duty assignment
VV	visual vigilance